REMARKS

Claims 23-31, 34-42 and 51 stand rejected in the 02/28/2007 Office action, and claims 32, 33 and 43-50 stand withdrawn in that Office action. Claims 23-51 are cancelled herein, and new claims 52 to 75 are added herein. The relationships of these claims are summarized in the following section.

Restriction/Election and Summary of Claim Relationships:

The 02/28/2007 Office action maintained the restriction based on lack of inventive step although acknowledging that the traversal arguments were persuasive as to the novelty of the technical feature linking the claims. However, in view of the dependency of the new claims, per below, and the amendments to the new independent claims, it is believed that this stated lack of inventive step is overcome. Accordingly, Applicant respectfully requests reconsideration of this restriction, believing further that it may be moot in view of the new claims are arguments herein.

Claims 52 and 53 directly derive from previously filed claims 23 and 24 however now limited to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7).

Claims 54 and 55 directly derive from previously filed claims 26 and 27 as well restricted to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7).

Claims 56-58 directly derive from previously filed claims 29-31 as well restricted to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7).

Claims 59 and 60 derive from previously filed claims 32 and 33, but are now restricted to be dependent from claim 52 and 59 respectively, therefore also restricted to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7).

Claims 61 and 62 directly derive from previously filed claims 34 and 35 and are now restricted to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7).

Claims 63-65 directly derive from previously filed claims 37-39 as well restricted to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7).

Claims 66 and 67 directly derive from previously filed claims 41 and 42 as well restricted to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7).

Claims 68-70 and 72-75 derive from previously filed claims 43-45 and 47-50, but are now restricted to be dependent from claim 61, therefore also restricted to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7).

Claim 71 derives from previously filed claim 51, also restricted to the 7S basic globulin sequences of the promoter and the leader (SEQ ID 6 and 7) and comprising steps as requested in a method claim.

The reasoning for now meeting the inventive step through the presentation of the claim set herein is provided in the following section.

Introductory Discussion of Distinctions in the Art Field:

Prior proceeding with the examination, the examiner is kindly requested to take into account the following considerations.

The examiner has raised an objection based on the fact that "7S soy globulin gene" regulating sequences were claimed, and that β-conglycinine and 7S soy globulin appear to be the same gene.

However, as well described through all the specification, as well as in the sequence listing, the SEQ IDs No 6 and 7, are found in the 7S soy **basic** globulin gene. This gene is completely different and has a completely different promoter, from the 7S soy globulin gene.

The prior art for the 7S globulins from soybean can be summarized as follows:

The leguminosae storage proteins are divided in two classes: globulins and lectins. The globulin class in turn is subdivided into two subclasses: legumins (11S hexameric proteins) and vicilins (7S trimeric or dimeric proteins).

β-conglycinine and **basic 7S globulin**, whose regulation elements were used to perform tissue-specific expression in seed (Sewry et al., 1995. The Plant Cell 7:945-956) belong to the vicillin subclass, but whereas the former was studied in detail, no detailed information is available on the 7S basic globulin functioning.

β-conglycinine is a storage protein of the soybean, consisting of three different subunits, α, α', β that interact non-covalently to form a trimer complex. The subunits are coded by a multigene family of 15 genes grouped in six nuclear DNA regions, whose expression is strictly regulated so as to be modulated during the plant life (Harada et al. 1989. The Plant Cell 1:415-425). Control is tissue-specific as well as stage-specific. In fact, the expression of each subunit is activated at high levels at the moment of embryo development, from the hearth shaped phase until complete ripening. The regulation of subunits α/α' and β expression occur at a transcriptional as well as post-transcriptional level (Harada et al. 1989. The Plant Cell 1:415-425), and the α' subunit, of 76 KDa, is accumulated more precociously and in a larger amount as compared to β subunit. This behavior is due to the greater strength of the α' subunit due to the presence of an enhancer region, absent in β, and of a sequence stabilizing the α' transcript.

7S basic globulin, is a storage protein of soybean, with a high methionine and cysteine content. It is completely different from β-conglycinin as also shown in the annexed sequence alignments (provided herein as Exhibits 1 and 2) that were made with the two programs BLAST 2 and MULTALIN with the two sequences downloaded from the genbank database. Consideration of this evidence is respectfully requested as to the issues discussed herein.

In both alignments, the first results are on the complete sequences, the second results are on the alignment of the promoters from position 1 to the ATG codon, the third results are on the alignment of the coding sequences.

Like β-conglycinine, the 7S basic globulin (Bg) also is stored in seed in large amounts. It consists of two subunits, one of 27 KDa, the other of 16 KDa encoded by the same mRNA . Bg is synthesized as sole precursor polypeptide consisting of a putative peptide signal and of two subunits. In the genome about four copies of the Bg gene are present (Watanabe and Hirano, 1994. Plant Phys. 105:1019-1020). This

protein is mainly located in the seed embryonal tissues and its expression pattern is unusual for a storage protein (Nishizava et al. 1994. Plant Cell Physiol. 35:1079-1085). This location suggests that the Bg is not a mere storage protein, having other functions as well. More accurate data on Bg location and expression period in soybean are not available.

SEQ ID NO:6 and SEQ ID NO:7 are the first example of the 7S basic globulin "family of genes" and more accurate data on Bg location and expression period in soybean are not available. In the prior art, it has never been verified whether the site- and time-specific expression mechanism of the 7S basic globulin is preserved in other transformed plant species (like tobacco), furthermore the prior art does not teaches that the 7S basic globulin from soybean is equivalent to β-conglycinin (see Watanabe and Hirano (1994), Harada (1989) and Nishizava (1994)). The prior art teaches that the 7S basic globulin (Bg) consists of two subunits, one of 27 KDa, the other of 16 KDa encoded by the same mRNA. Bg is synthesized as sole precursor polypeptide consisting of a putative peptide signal and of two subunits. In the genome about four copies of the Bg gene are present (Watanabe and Hirano, 1994. Plant Phys. 105:1019-1020).

Thus, the promoter SEQ ID NO:6 and the signal sequence SEQ ID NO:7 are different, both at level of expression pattern and tissue accumulation (see Watanabe and Hirano (1994), Harada (1989) and Nishizava (1994)), from the well described β-conglycinin. It would be, very difficult for the skilled person, to predict whether the 7S basic globulin regulating elements could provide for the in seed expression of lysosomal enzymes in active form, as there are no indications in the prior art that expression vectors comprising the said regulating elements could provide expression of active enzymes in plant.

Objections to the Specification:

Applicants have amended the noted paragraphs of the specification, and the Abstract, to overcome the objections. Also, a similar inadvertent symbol error was noted in paragraph 0122 beginning on page 28, line 21 and this is corrected herein.

Applicants respectfully request withdrawal of the objections.

Objections to the Claims:

Applicants have incorporated the requested changes to the new claims where appropriate, noting however that some of the requested changes to claims 23 and 34 (see new claims 52 and 61) were not added based on other changes to these claims. However, the objected to language is no longer present. Applicants respectfully request withdrawal of these objections where such objections are not moot.

Rejection of the previously filed claims under 35 USC §112 and 101.

In compliance with the objections raised by the examiner concerning the failure to comply with the written description requirement, all the presently filed claims have been drafted taking into account the said objections. The claims have thus been amended in order be restricted to the embodiment wherein SEQ ID No 6 and 7 are used as regulating elements for the carrying out of the invention. The claims presently filed, hence, comply with the requirement of 35USC §§ 112 and 101. Withdrawal of these claim rejections is respectfully requested.

Rejection of the previously filed claims under 35 USC §103.

Although acknowledging the compliance with the Novelty requirements of the previously filed claims, the said claims have been rejected by the examiner for lack of inventiveness based on the fact that Radin et al. allegedly teach transgenic tobacco plants expressing lysosomal enzymes and seeds of such plants.

The examiner also points out that Radin et al. do not teach the regulating sequences of 7S soy globulin nor an expression amount in seed of at least 0.8% of the total proteins of the seed.

The examiner also cites Chen et al, for the teaching of constructs comprising different fragments of the promoter from the α' subunit gene being a 7S globulin protein and transgenic plants using the said promoter for a high accumulation in seed of certain proteins.

First of all it is herein noted that, although Radin et al. describes the expression of some lysosomal enzymes in tobacco plants, these enzymes are **produced essentially in leaf** by plants transformed via the use of vectors containing the MeGa promoter or the cauliflower mosaic virus 35S promoter.
Although allegedly claiming also in-seed expression with the 35S promoter or with several inducible promoters, only demonstrates the use of the sole MeGa promoter for the in-leaf expression of GC and never demonstrates the possibility of an in-seed expression of said enzymes. The promoters cited by Radin et al, however, are not functional in-seed (as also proved by the applicant for the 35S promoter in figure 12 for the present application).
Hence, the document of Radin et al. does not teach the expression of lysosomal enzymes in seeds, as it does not even teach a promoter of a plant gene specific for the expression in seed storage organs and stage specific, nor does it teach an in seed (as it does not teach in seed expression) expression in an amount of at least 0.8% of total proteins of the seed, nor does it teach or even suggest: a promoter from a 7S soy globulin gene or a signal sequence from a 7S soy globulin protein, a promoter from a soy 7S basic globulin gene or a signal sequence from a soy 7S basic globulin protein.

Secondly, Chen at al, although teaching the expression pattern of the β-conglycinine α' subunit protein in transgenic plants and its high expression level (0.1-5% of the proteins extracted from the seeds), also demonstrates at the same time (cfr. page 115 column 2 lines) that the β subunit of the same protein has an expression pattern 10-15 fold less than the α' subunit of the same protein, in contrast with the β subunit of the phaseolin gene, thus pointing out the unpredictability of the expression pattern among 7S globulin genes.
An expression of 10 fold less would be 0.01-0.5% of the proteins extracted from the seeds and an expression of 15 fold less would be 0.005-0.25% of the proteins extracted from the seeds, which is well below the "at least 0.8%" claimed in the present application.
The paper published by Chen et al., hence, shows that "equivalent" subunits of

similar genes do not provide for the same expression patterns as it would be expected.
The paper is silent on the expression or on the possibility of the expression in seed of **active enzymes** using β conglycinine promoters.

The mere expression study and the localization of a seed protein in seeds of transgenic plants does not give guidance to the person skilled in the art for the possibility of using regulating elements of the said proteins for the expression of mammalian enzymes in an active form in seed.

The Chen et al. paper is also silent about the expression patterns and about the identification of the regulating sequences of the soy 7S basic globulin regulating regions

Whitelam discusses "bio-farming" in general and teaches that there are advantages to seed-localized expression of recombinant proteins but, at the time the application was filed, the use of specific promoters able to determine high levels of recombinant protein accumulation in specific compartments of the seed, stability of the protein and post-translational modifications was not obvious also to the person skilled in the art as above discussed.
In fact, Whitelam basically poses the problem solved by the present invention.

There were, at the time of filing of the application
a. no teachings of the promoters available for an in seed highly efficient expression of enzymes
b. no teachings that the soy 7S basic globulin promoter and leader sequences could have provided an efficient system in order to solve the problem posed by the Whitelam publication.

The unpredictability of the expression pattern of "equivalent" regions of similar genes highlighted by Chen et al, does not allow the person skilled in the art to expect the results obtained by the applicant by using soy 7S basic globulin

regulating elements.

Furthermore, the lack of knowledge of the seed compartment wherein the leader sequence of the said gene vehicles the protein, did not allow the skilled person to consider obvious the expression of an heterologous enzyme in active form using the said sequence as regulating element for the expression of the said enzyme.

Moreover, the paper of Chang et al, proves that the skilled person cannot predict the expression levels of vincillin genes only on data regarding the expression pattern of genes or subunits of genes of the same family.

The soy 7S basic globulin, furthermore, is structurally different for the 7S soy genes, rendering any prediction even more impossible to make.

Based on the above arguments, it is herein believed that the presently filed claims cannot be considered obvious on the basis of the prior art cited and that they fulfill the requirements of 35 USC § 103 and that a patent should be granted with the said claims.

Applicants therefore respectfully request withdrawal of all claim rejections.

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Having overcome all rejections and objections, Applicants respectfully request that a timely Notice of Allowance be issued in this case.

The Examiner is invited to call the undersigned if clarification is needed on any aspects of this Reply/Amendment, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

Joseph Fischer
Patent Attorney, Registration No. 51,210
(Customer No. 29,391)
Mailing Address and Phone/Fax numbers:
Beusse Wolter Sanks Mora & Maire, P.A.
390 N. Orange Avenue, Suite 2500
Orlando, FL 32801
Telephone: 407-497-7437
Fax: 407-926-7720

EXHIBIT 1

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.16 [Mar-25-2007]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 0 expect: 10.0000 wordsize: 11 Filter ☑ View option Standard
Masking character option X for protein, n for nucleotide Masking color option Black
☐ Show CDS translation Align

Sequence 1: lcl|seq_1
Length = 3393

Sequence 2: lcl|seq_2
Length = 3636

No significant similarity was found

```
CPU time:      0.03 user secs.      0.00 sys. secs          0.03 total secs.
```

SEQUENCE 1 = D16107 complete GenBank accession
SEQUENCE 2 = M13759 complete GenBank accession

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.16 [Mar-25-2007]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 0 expect: 10.0000 wordsize: 11 Filter ☑ View option Standard
Masking character option X for protein, n for nucleotide Masking color option Black
☐ Show CDS translation Align

Sequence 1: lcl|seq_1
Length = 1428

Sequence 2: lcl|seq_2
Length = 962

No significant similarity was found

```
CPU time:      0.03 user secs.      0.01 sys. secs          0.04 total secs.
```

SEQUENCE 1 = D16107 from 1 to ATG
SEQUENCE 2 = M13759 from 1 to ATG

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.16 [Mar-25-2007]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 0 expect: 10.0000 wordsize: 11 Filter ☑ View option Standard
Masking character option X for protein, n for nucleotide Masking color option Black
☐ Show CDS translation Align

Sequence 1: lcl|seq_1
Length = 1284

Sequence 2: lcl|seq_2
Length = 1920

No significant similarity was found

```
CPU time:      0.02 user secs.      0.02 sys. secs          0.04 total secs.
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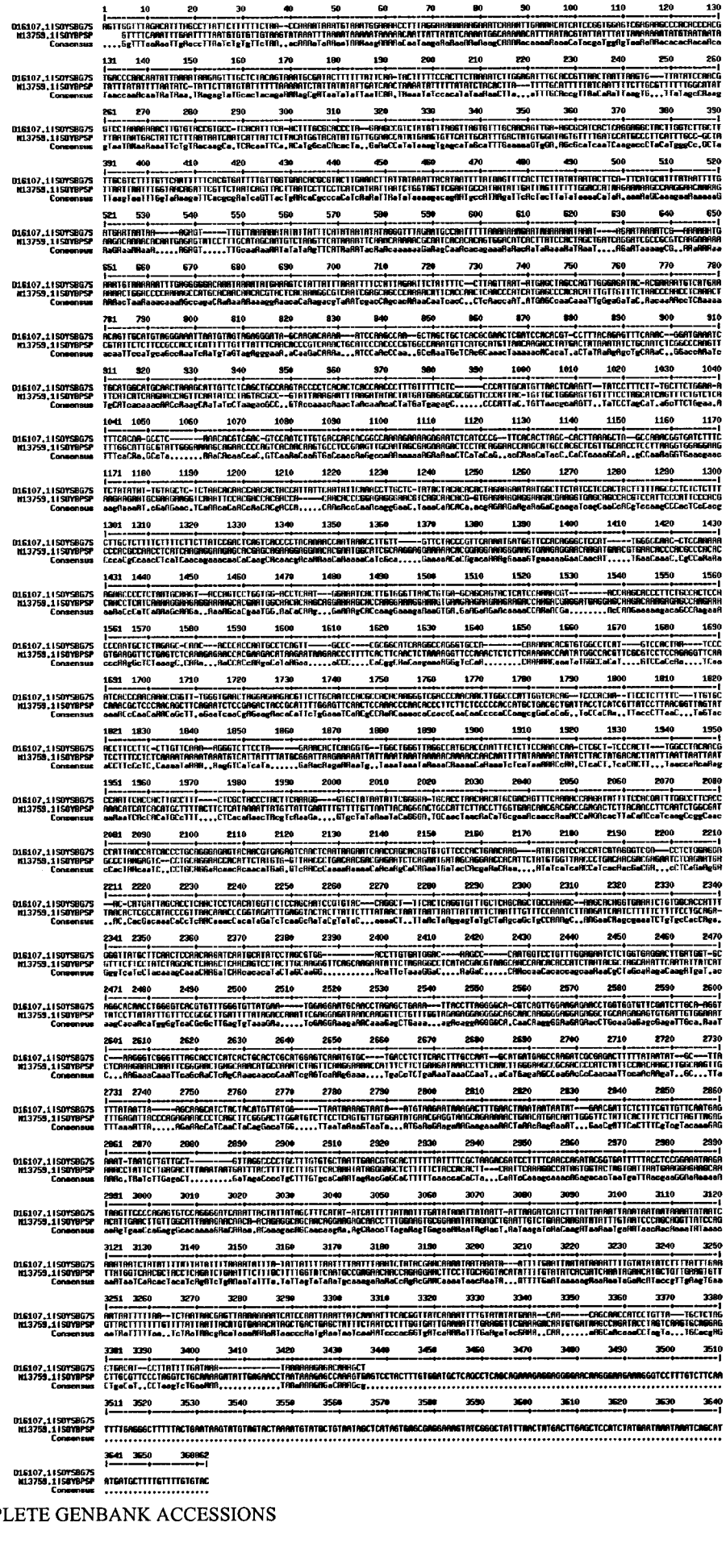
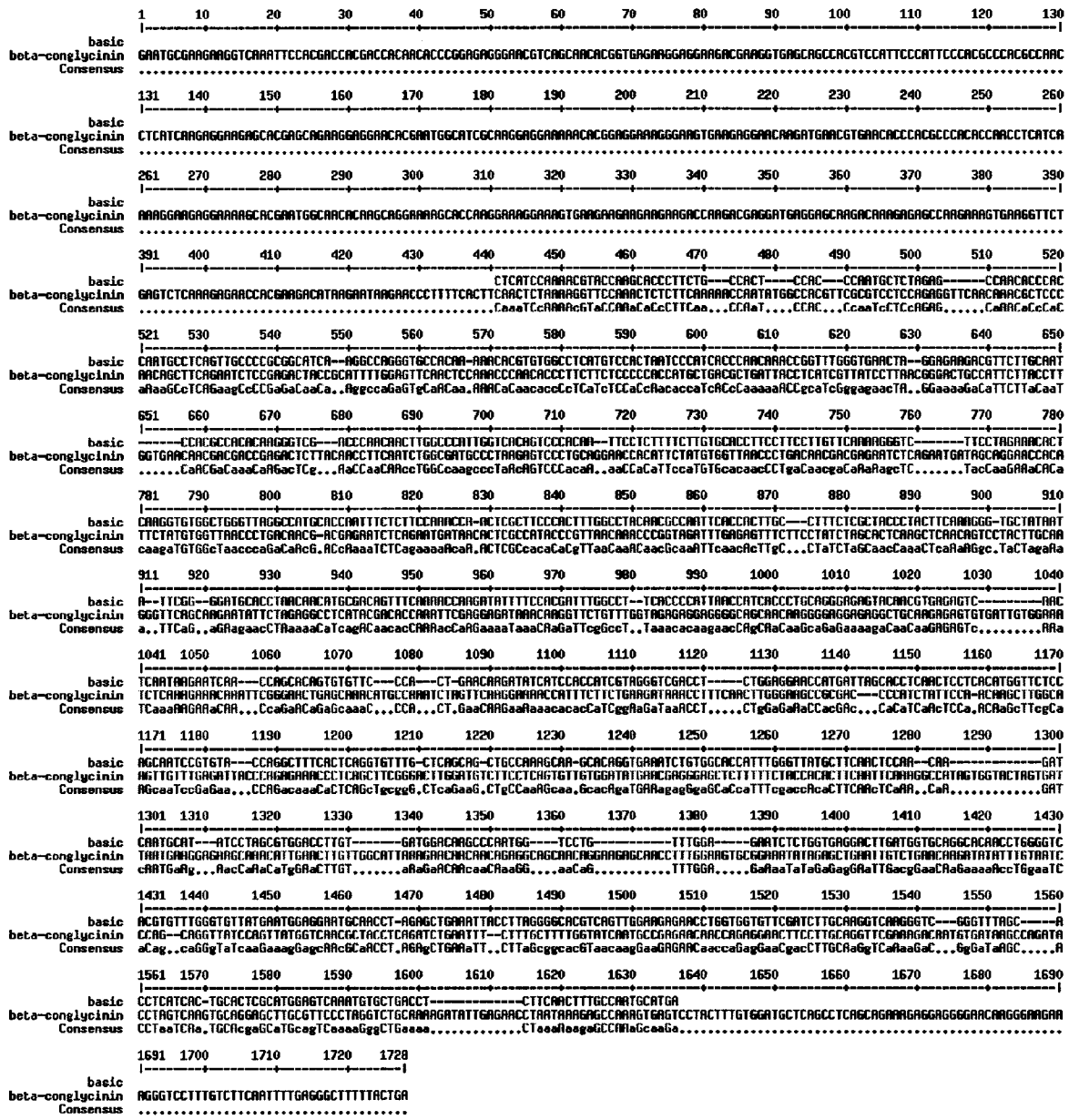
SEQUENCE 1 = D16107 coding sequence
SEQUENCE 2 = M13759 coding sequence

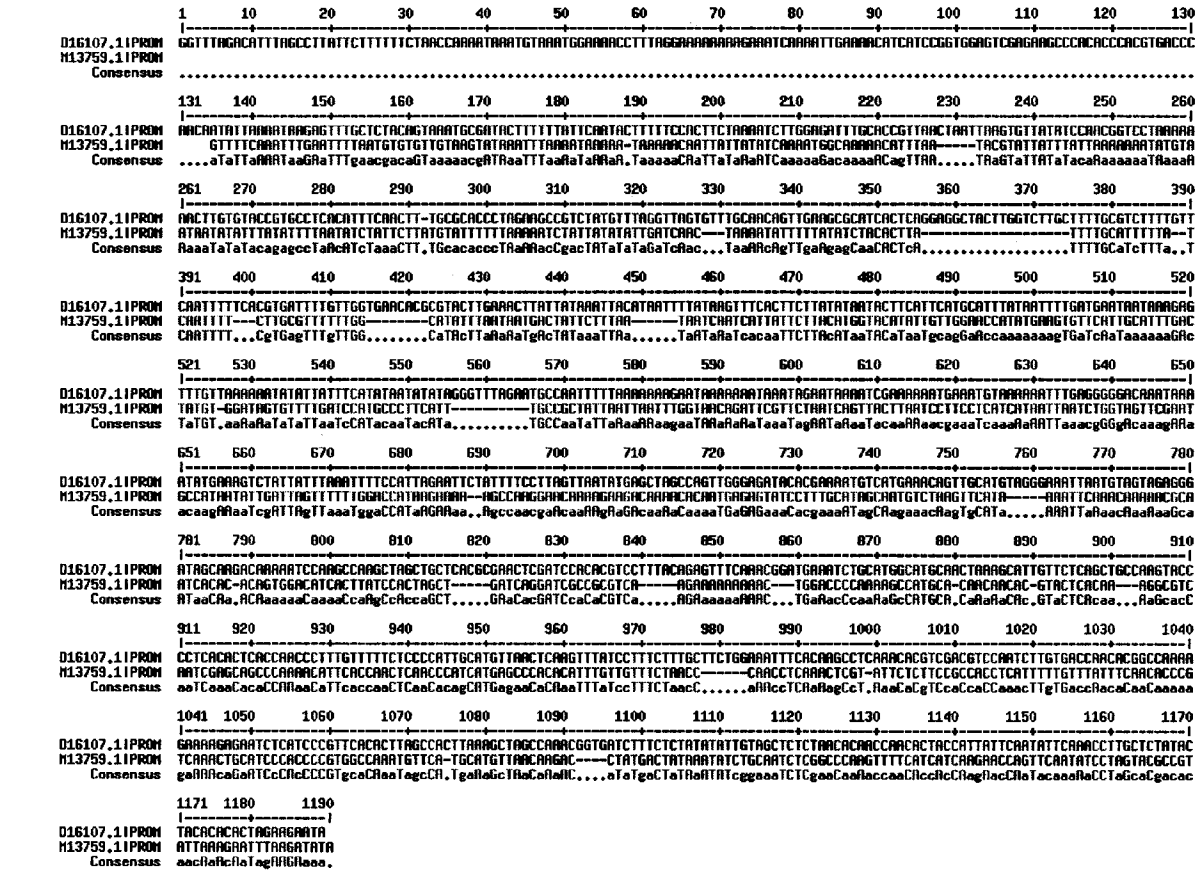
EXHIBIT 2



COMPLETE GENBANK ACCESSIONS



CODING SEQUENCE: Basic (D16107); beta-conglycinin (M13759)



FROM 1 TO ATG